ARTICLE IN PRESS

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
xx (xx): xx-xx, 2024
DOI: 10.9775/kvfd.2024.32618

RESEARCH ARTICLE

Evaluating *Linum usitatissimum* Seeds Extract as Potential Alternative Biochemical and Therapeutic Agent Against Induced Coccidiosis in Broiler Chicken

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How to cite this article?

Hussain K, Abbas A, Rehman A, Waqas MU, Ahmad B, Mughal MAS, Abbas RZ, Zaman MA, Khan JA, Raza MA: Evaluating *Linum usitatissimum* seeds extract as potential alternative biochemical and therapeutic agent against induced coccidiosis in broiler chicken.. *Kafkas Univ Vet Fak Derg*, 2024 (Article in Press).
DOI: 10.9775/kvfd.2024.32618

Article ID: KVFD-2024-32618
Received: 06.07.2024
Accepted: 20.09.2024
Published Online: 03.10.2024

Abstract

Coccidiosis is a significant disease of poultry and is usually treated using various synthetic anticoccidial drugs. However, the effectiveness of this approach has been compromised due to drug resistance. Medicinal plants are being considered as potential alternatives to these drugs. As part of ongoing research, an investigation was conducted to assess the anticoccidial potential of *Linum usitatissimum* seed extract (LUE) and its impact on hematological parameters in broiler chickens with experimental coccidiosis. A total of 108 broiler chicks were divided into six groups. The chicks in Groups I, II, and III were given plant extract at three different concentrations: 4%, 5%, and 6%, respectively, at one week of age. Group IV was the positive control - these chickens were treated with toltrazuril. Group V served as the negative control, meaning they were infected but not given any medication. Group VI was the normal control group. All groups, except Group VI, were orally infected with 60,000 sporulated oocysts when they were 18 days old. After 7 days of giving orally infection, six birds from each group were euthanized so that we could examine their feed conversion ratio (FCR), oocyst score, lesion score, fecal score, serum chemistry and hematology. The results showed that the *L. usitatissimum* extract exhibited anticoccidial activity. It improved the FCR and reduced lesion, oocyst, fecal scores and blood and serum chemistry.

Keywords: Anticoccidial, Chicken, Coccidiosis, *Linum usitatissimum*

Introduction

Protozoa of the genus *Eimeria*, single-cell parasites from the phylum Apicomplexa, with complicated and several life cycle phases, cause avian coccidiosis [1]. *Eimeria* species mainly affect avian intestines, causing symptoms such as bloody diarrhea, poor feed conversion ratio (FCR), and even death. This results in considerable financial losses for chicken farming. *E. tenella* is one of the most harmful *Eimeria* species, causing cecal coccidiosis in hens. Each year, the poultry sector suffers significant financial losses due to coccidiosis, which starts with the intake of sporulated oocysts [2]. Due to quick sporulation process, naturally occurring coccidian oocysts abound and generate millions of oocysts [3]. Although Sulfanilamide was first anticoccidial utilized as a treatment against coccidiosis in poultry, a range of anticoccidial feed additives and antibiotics have also been developed and used. Synthetic chemicals and anti-coccidials are usually employed to control coccidiosis. However, reasonable use of anticoccidial medications resistance has evolved [4]. Furthermore, time is needed to locate some alternative tools for efficient coccidiosis control. Based on their Therapeutic and immunomodulatory activity,

various plants were documented as anticoccidial and immunomodulatory over the past ten years [5-7]. Using antioxidant-rich and biological active plant extracts has become especially important in view of resistance to synthetic antibiotics, phenols, flavonoids, tannins and saponins are being used as an alternate approach to treat coccidiosis [8,9]. Like other plant with anthelmintic and therapeutic activity, *Linum usitatissimum* often referred as linseed and locally known Alsi contains numerous pharmacological properties against different parasitic and bacterial infections due to its diverse antioxidant chemicals [10]. Therefore, the present study was designed to evaluate the anticoccidial capacity of *L. usitatissimum* seed against induced infection in chickens which was caused by *Eimeria* based on existing literature including antioxidant properties.

Material and Methods

Ethical Approval

The research is conducted by the approval from ethical committee of University of Agriculture Faisalabad under the PSF, Project No. 185 and PARB, Project No. 358 (No. 628/6-08-2013)

Plants Material

After procuring *L. usitatissimum* (Alsi) seeds from the local market and they were crushed using a grinder machine. Afterwards, we prepared an aqueous methanolic extract of *L. usitatissimum* using Soxhlet's apparatus (Velp Italy) following the method described by Abbas et al.[11]. The suspension was evaporated in a rotary evaporator (Heidolph Germany) at a temperature not exceeding 50°C. The prepared *L. usitatissimum* extract was then stored in a refrigerator at 4°C after freeze drying until further use.

Parasite

The parasite material was collected from outbreak cases in Faisalabad as well as the intestines of naturally infected chickens with *Eimeria*. To induce sporulation, the material was immersed in a potassium dichromate solution (2.5%) at 25-29°C and 60-80% humidity. The sample was next inspected under a microscope [12].

The number of sporulated oocysts was calculated using the Modified McMaster procedure. The material was put into the chambers of the McMaster slide and left alone for 2-3 min to let the sporulated oocysts to float and become visible. The slide was next examined under a microscope at low (10x) and high (40x) magnification

Experimental Design

A total of 108 broiler chicks were obtained and divided into six groups, with 18 birds in each group. Groups I, II, and III were given plant extract at three different concentrations (4%, 5%, and 6%, respectively) at one week of age. Group IV was kept as the positive control (infected plus toltrazuril® from A&K Pharmaceuticals, Faisalabad, provided at a rate of 1 mL/L of water). Group V served as the negative control (infected and non-medicated), and group VI as the normal control (non-infected and non-medicated). By the time the chickens reached eighteen days, all groups except group VI had been orally infected with 60.000 sporulated oocysts. Six birds from each group were sacrificed seven days after the inoculation to collect data on FCR, oocyst score, lesion score, fecal score, serum chemistry, and hematology.

Evaluation of Anticoccidial Activity

The potential of (LUE) as an anticoccidial treatment was investigated using metrics such as (FCR), lesion score [13] and oocyst score [14]. The fecal scores of birds in each group were monitored to assess the severity of illness at day 3 to 7. To evaluate the fecal score in chickens, a standard method is used. The optimal time to assess the emergence of illness is between the third and seventh days after inoculation. The fecal score chart ranges from 1 to 5, representing increasing degrees of disease progression [15].

Hematological Parameters

The collected blood samples were tested for packed cell volume (PCV) by following the microhematocrit method with slight modification. Hemoglobin level (Hb) was determined using Sahli's device. Erythrocyte and leukocyte counts were performed using a hemocytometer using Natt and Herrick solution under compound microscope at 10x.

Serum Chemistry

The plant extract was evaluated for toxic possession, cellular injury, and serum samples using various imported assays (Merck, Germany) to determine the levels of serum enzymes (LAT, LDH, Creatinine) [16].

Statistical Analysis

Statistical analysis was performed using the ANOVA approach and SAS statistical analysis software version 9 [17]. The data was considered statistically significant with a P value <0.05.

Results

All the groups offered with *L. usitatissimum* extract (LUE) revealed improved FCR at classified doses in *Table 1*. However, the admirable result showed by the group which was administered with higher dose and the results were similar ($P>0.05$) to standard medicine (Toltrazuril®)

A lower lesion score at graded doses was observed in (LUE) treated group in *Table 2*. However, the admirable result showed by the group which was administered with

Table 1. *Feed conversion ratio (FCR) of plant extract treated groups*

Groups	Feed Consumed (g)	Ending Weight (g)	Feed Conversion Ratio (g/g)*
L. usitatissimum 4%	920.1	418.18	2.20
L. usitatissimum 5%	990.2	405.45	2.44
L. usitatissimum 6%	941.5	408.90	2.30
Positive control	910.16	409.86	2.22
Negative control	964.4	368.40	2.61
Normal Control	916.6	422.6	2.16

**Due to feeding in group statistical analysis was not achievable because FCR is simple ratio*

Table 2. *Lesion score of different plant extract treated groups*

Groups	0	+1	+2	+3	+4	Mean
L. usitatissimum 4%	0	1	2	2	1	1.83 ± 0.54^{b}
L. usitatissimum 5%	0	2	2	2	0	1.50 ± 0.51^{c}
L. usitatissimum 6%	0	3	3	0	0	1.66 ± 0.83^{bc}
Positive control	1	3	2	0	0	1.33 ± 0.40^{d}
Negative control	0	0	0	3	3	3.33 ± 0.51^{a}
Normal Control	0	0	0	0	0	0

Means with different superscripts are significantly different (P<0.05) from each other
+1: No lesions, +2: Very few, +3: Large amount, +4: Blood and Death

Table 3. *Oocyst score of treated groups with plant extract*

Groups	0	+1	+2	+3	+4	+5	Mean
L. usitatissimum 4%	0	1	3	1	1	0	2.00 ± 0.75^{b}
L. usitatissimum 5%	0	2	2	2	0	0	1.50 ± 0.51^{c}
L. usitatissimum 6%	0	3	3	0	0	0	1.66 ± 0.54^{bc}
Positive control	0	2	3	1	0	0	1.66 ± 0.75^{bc}
Negative control	0	0	0	3	2	1	3.83 ± 0.40^{a}
Normal Control	–	–	–	–	–	–	–

Means with different superscripts are significantly different (P<0.05) from each other
0: No oocyst, +1: 1-10 oocyst/Field, +2: 11-20 oocyst/Field, +3: 21-50 oocyst/Field, +4: 51-100 oocyst/Field, +5: More than 100 oocyst/Field

Table 4. *Fecal score of groups treated with Plant Extract*

Groups	Day 3rd	Day 4th	Day 5th	Day 6th	Day 7th
L. usitatissimum 4%	–	2.04 ± 0.75^{ab}	2.15 ± 0.75^{b}	1.12 ± 0.63^{b}	–
L. usitatissimum 5%	–	1.34 ± 0.51^{b}	2.01 ± 0.63^{b}	1.52 ± 0.54^{b}	–
L. usitatissimum 6%	–	1.52 ± 0.54^{ab}	1.68 ± 0.51^{b}	1.51 ± 0.54^{b}	–
Positive control	–	1.51 ± 0.83^{ab}	1.68 ± 0.51^{b}	1.34 ± 0.51^{b}	–
Negative control	–	2.68 ± 1.40^{a}	2.99 ± 0.42^{a}	2.71 ± 0.79^{a}	–
Normal Control	–	–	–	–	–

Means with different superscripts are significantly different (P<0.05) from each other

Table 5. *Serum enzymes values of groups treated Plant Extract (Mean±SD)*

Groups	ALT	ASAT	LDH	Urea	Creatinine
L. usitatissimum 4%	9.46±0.91^{b}	180.72±10.08^{b}	476.01±16.91^{b}	5.40±0.81^{b}	0.21±0.02^{b}
L. usitatissimum 5%	9.57±0.97^{b}	182.60±12.14^{b}	483.95±21.52^{b}	5.55±0.97^{b}	0.18±0.02^{b}
L. usitatissimum 6%	9.74±1.38^{b}	177.55±14.72^{b}	477.24±22.21^{b}	5.60±1.02^{b}	0.19±0.03^{b}
Positive control	9.69±1.15^{b}	181.85±10.15^{b}	477.42±21.15^{b}	5.20±0.48^{b}	0.19±0.02^{b}
Negative control	24.62±2.31^{a}	288.87±36.21^{a}	891.96±22.16^{a}	20.60±1.12^{a}	0.70±0.03^{a}
Normal Control	8.98±1.78^{b}	195.03±13.46^{b}	471.45±15.78^{b}	5.45±0.59^{b}	0.16±0.02^{b}

Means with different superscripts are significantly different (P<0.05) from each other

Table 6. *Hematological values o of groups treated Plant Extract (Mean±SD)*

Groups	PCV %	Hb g/dL	RBC 10^{6}/µL	WBC 10^{3}/µL
L. usitatissimum 4%	29.14±1.67^{a}	10.22±1.06^{a}	3.99±0.76^{a}	21.67±2.93^{b}
L. usitatissimum 5%	28.20±1.61^{a}	11.43±1.35^{a}	3.64±0.86^{a}	22.74±2.70^{b}
L. usitatissimum 6%	27.01±1.66^{a}	11.81±1.28^{a}	3.41±0.71^{a}	21.66±1.64^{b}
Positive control	24.17±2.14^{a}	11.20±0.64^{a}	3.27±0.71^{b}	21.41±2.78^{b}
Negative control	20.15±1.15^{b}	8.91±0.82^{b}	1.88±0.12^{c}	34.01±5.04^{a}
Normal Control	25.34±1.03^{a}	11.0±1.34^{a}	3.15±0.58^{ab}	22.51±3.26^{b}

Means with different superscripts are significantly different (P<0.05) from each other

a higher dose and the results were similar (P>0.05) to standard medicine (Toltrazuril®)

All groups treated with *L. usitatissimum* extract (LUE) showed minimal oocyst scores at graded doses in *Table 3*. However, the admirable result showed by the group which was administered with higher dose and the results were similar (P<0.05) to standard medicine (Toltrazuril®)

L. usitatissimum extract (LUE) administered groups showed minimal fecal score at graded doses in *Table 4*. However, the admirable result showed by the group which was administered with higher dose and the results were similar (P<0.05) to standard medicine (Toltrazuril®)

Minimal serum enzyme values in all (LUE) administered groups were observed in *Table 5*. However, the admirable result showed by the group which was administered with higher dose and the results were similar (P>0.05) to standard medicine (Toltrazuril®)

Maximum hematological (PCV, Hb, RBCs and WBCs) values were observed in *Table 6*. However, the admirable result showed by the group which was administered with higherdose and the results were similar (P>0.05) to standard medicine (Toltrazuril®)

Discussion

Recent reports have explored alternative methods for treating coccidiosis by using plant antioxidant composites such as phenols, flavonoids, tannins, and saponins [1,4,11,18]. This investigation found that *L. usitatissimum* shows anticoccidial activity and has positive effects on serum chemistry, hematological values, oocyst score, lesion score, and fecal score. The results were identical to those of accomplished with the traditional medication (Toltrazuril®). Specifically, *L. usitatissimum*, when administered at doses of 4%, 5%, and 6%, confirmed a dose-dependent potential to combat mixed *Eimeria* infections. The maximum concentration confirmed results corresponding to the toltrazuril® dealt with group (P>0.05). Previous studies inspecting the capacity of various herbal extracts to combat coccidiosis have also mentioned similar findings, with the effectiveness of the extracts varying depending on the dosage administered [19-21].

In a study, the weight gains in broiler chicks infected with *Eimeria* was significantly reduced by an ethanolic extract from Carica papaya leaves [22]. Studies showed that orally administered *Ageratum conyzoides* extract to 28-day-old chicks increased the number of red blood cells (RBCs), white blood cells (WBCs), and PCV in the treated birds. This eventually resulted in a decrease in infection levels and excretion of oocysts. The antioxidant chemicals found in *A. conyzoides*, including flavonoids, phenols, conyzorium, methexnebilitin, and quercetin, may reduce the oxidative stress caused by coccidiosis. Likewise, *A. conyzoides* has been found to have anticoccidial properties. This means that it can diminish *Eimeria* infection by inquisitive with lipid peroxidation [8,23]. The study found that the groups given the plant extract had lower levels of certain serum

enzymes similar to the control groups, indicating that the extract did not have any harmful effects. Hepatotoxicity can be determined by looking at the levels of AST and ALT, while nephrotoxicity can be determined by studying the levels of serum creatinine and urea.

A recent study showed that pulp of olive fruit when combine with vitamin C gives positive results on hatchability, weight gain, and improved growth rate [24]. Recent study of catechin *Uncaria gambir* extract which is planned on broiler birds to see meat quality, growth rate, serum /Plasma values and also antioxidant activity gives favorable results [25].

There are some other studies which showed the antioxidant, biomedical, immunomodulatory, antibacterial, resistant free effects of plant material, zinc oxide, Sodium Alginate and nanoparticles on animals [26].

The findings mentioned above indicate that plant-derived extracts could effectively help in dealing with chicken coccidiosis and its associated toxicity. These results also highlight the potential of *L. usitatissimum* as a safe and herbal anticoccidial agent, which warrants further research into the plant's active substances and modes of action. By incorporating *L. usitatissimum* into chicken health management, the risk of drug resistance may be reduced, and overall chicken health may be improved. This could present a sustainable and successful alternative to synthetic anticoccidial medicines

Declarations

Availability of Data and Materials: Research data will be provided by the author (Kashif Hussain) on request.

Acknowledgements: Support for this study was provided by the Punjab Agricultural Research Board, Project No. 358 (Assessment and Advancement of herbal anticoccidials for the management of coccidiosis in poultry) and the Pakistan Science Foundation, Project No. 185.

Funding Support: The Main Researcher recognizes the financial help for this research from Pakistan Science Foundation, Project No. 185.

Competing Interest: There is no conflict of interest between all the author

Declaration of Generative Artificial Intelligence (AI): This whole article is free from any AI tool.

Author Contributions: KH apprehended and planned the study; AA, AR, MUW, BA, MASM did work on methodology and RZA, MAZ, JAK, MAR and investigated the data and help in the writeup of the manuscript

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